On the basis of the outlined *in vivo* studies, the current study by Ren and co-workers,[1] and the established vascular renal effects of adenosine,[10] a concept could be envisioned in which small perturbations of the TGF signal from the operating point trigger parallel changes in local adenosine concentrations that induce primarily afferent arteriolar vasoconstriction via adenosine A_1 receptors and efferent vasoconstriction due to passive interactions with R_A (see above) and/or due to some coactivation of adenosine A_1 receptors on the efferent arteriole such that P_{GC} remains unchanged. In comparison, more excessive TGF signals trigger larger local adenosine concentrations that in addition induce a vasodilating influence on the efferent arteriole via adenosine A_2 receptors, which attenuates or prevents a further rise in R_E and thus lowers P_{GC} (Figure 1).

In summary, the basal conditions, the co-response of the afferent arteriole, and the strength of TGF stimuli applied may dictate the net magnitude and even the direction of the efferent arteriolar response. The study by Ren and co-workers[1] has defined an important influence, which tends to reduce R_E during TGF activation, and this appears to be another important effect of local adenosine. From a teleological standpoint, a TGF-induced adenosine-mediated efferent vasodilatory influence would be appealing especially in deep nephrons, where the postglomerular blood flow is nutritive for the medulla and thus consistent with a proposed role of adenosine in metabolic control of kidney function.[10]

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Diabetes: Caught in the Akt?

PM Price[1]

One complication of diabetes is a pronounced renal cellular hypertrophy, inevitably resulting in chronic fibrotic changes. Chuang and colleagues demonstrate that hypertrophy *in vitro* is dependent on an increased phosphoinositide 3-kinase (PI3K) activity and is correlated with increased levels of $p21^{WAF1/Cip1}$, a cell-cycle regulator that was previously associated with renal fibrosis and sclerosis from nondiabetic causes.

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The response of hypertrophy occurs in organs, such as heart, lungs, and kidneys, usually in reaction to a demand for increased work load. This reaction, in which there is an increase in total cell volume without a similar increase in total cell number, has been suggested in older literature to be maladaptive.[1] Renal hypertrophy is found in several different disorders and is associated with a progressive loss of kidney function and eventual glomerular and tubulointerstitial fibrosis. Although hypertrophy may be accompanied or preceded by a period of hyperplasia,[2–4] chronic fibrotic changes in the kidney are preceded by hypertrophic growth.[5] Several *in vivo* models, such as the rodent models of streptozotocin-induced diabetes and renal ablation, mimic clinically observed kidney and systemic changes. A similar hypertrophic response was also observed *in vitro* when kidney cells were cultured in high-glucose-containing medium.[6–8]

The commitment to hypertrophic growth by kidney cells in reaction to diabetes or ablation has recently been approached by the use of gene knockout studies. Early evidence had shown that proteins associated with cell-cycle regulation and inhibition were increased in the kidney after both acute and chronic stress,[9,10] and that these inhibitors, transduced into kidney proximal tubular cells, caused hypertrophy.[11] In knockout models of one of these proteins, the p21 cyclin-dependent kinase inhibitor, hypertrophy, glomerulosclerosis, and interstitial fibrosis did not develop after partial renal ablation,[4] and glomerular hypertrophy did not occur in streptozotocin-treated mice.[12] Thus it may be concluded that the hyperplastic increase in kidney cells observed in these models was not detrimental, but that hypertrophic

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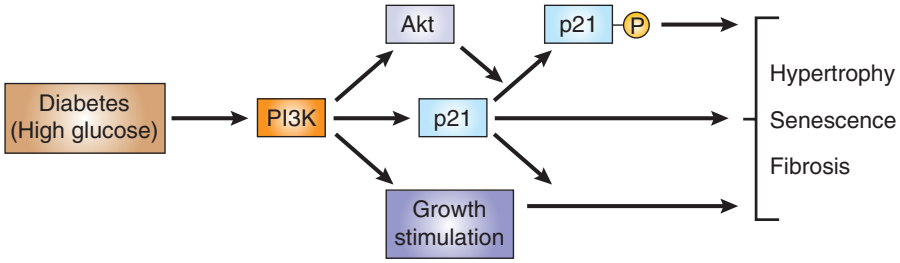

Figure 1 | Interactions of Akt, p21, and P13K after high glucose stress. In diabetes (or in the high-glucose *in vitro* model used by Chuang *et al.*[13]), phosphoinositide 3-kinase (PI3K) is upregulated, resulting in elevated levels of Akt and p21. Growth is stimulated by PI3K but could also be stimulated by other types of renal stress. Several of the possible mechanisms for hypertrophic growth under these conditions are discussed in this Commentary. First, persistent growth stimulation and elevated cell-cycle inhibitor levels could result in hypertrophy rather than hyperplasia. Second, Akt-dependent p21 phosphorylation could sequester p21 in the cytoplasm, where it may also stimulate growth. Third, p21 by itself is known to activate pathways of senescence, which may lead to fibrotic changes associated with aging.

growth was maladaptive. Preventive intervention in the p21-induced hypertrophic response may be possible if this pathway can be further elucidated and potential targets identified.

Chuang *et al.*[13] (this issue), using LLC-PK1 cells, a porcine kidney proximal tubule-derived cell line, show that the hypertrophic response to culture in high-glucose medium is dependent on an increase in phosphoinositide 3-kinase (PI3K) activity, and as in other studies, hypertrophy was also associated with elevated p21. Inhibition of PI3K not only ameliorated hypertrophy but also lowered p21 levels. So can these two proteins, one an inhibitor of cell-cycle kinases, and the other a kinase activator of the oncoprotein Akt, have a common pathway that ultimately results in cellular hypertrophy?

More than 500 protein kinases are encoded by the human genome. The functions of many are known, and these enzymes control protein expression and degradation and protein function, localization, and interactions. These kinases direct the function of the cell and, ultimately, its life and death. It is not surprising, therefore, that the fate of cells in the kidney, and the fate of the kidney as an organ, are also controlled by protein kinases.

The p21 protein is a member of a family of proteins that inhibit the cyclin-dependent kinases responsible for progression through DNA synthesis and mitosis during the cell cycle. It is constitutively expressed at low levels and is usually localized to the nucleus. Its inhibitory activity and the finding that p21 is highly upregulated after DNA damage and other types of cellular stress led to the proposal that these inhibitors control the cell cycle, either allowing or inhibiting cell replication. The 'regulation' imposed on the cell cycle by p21 makes it possible for cells under various types of stress, many of which elevate p21, to interrupt replication and division until the stress is relieved and p21 returns to lower levels. On the other hand, the upregulation of p21 could work against this system unless the load is reduced. The inability to undergo cell division coupled with the stress imposed on an organ by increased work load may result in increased size by hypertrophy. This reaction is likely to be regulated by both the cell type and the extent of stress encountered.

PI3K promotes cell-cycle activity by several different pathways, including inhibition of proteins that repress cell division and activation of proteins that stimulate replication. After activation, PI3K phosphorylates a phosphoinositide intermediate, allowing Akt, a downstream effector of PI3K, to be recruited and activated. Although many pathways are initiated by PI3K, the Akt pathway in particular is relevant to cell growth. As was first noted in *Drosophila*,[14] overexpression of Dakt1 (the fly homologue of human Akt) caused cellular and organ hypertrophy, whereas disruption of the Dakt1 gene caused a significant decrease in size. This effect was also noted in knockout and overexpressing transgenic mice (cited by Shiojima *et al.*[15]), even though the Akt gene in higher vertebrates is redundant (there are three Akt genes), and the effect of loss of one gene can be at least partially compensated for by the other genes.

Chuang *et al.*[13] correlated the activation of PI3K, Akt, and elevated p21 with hypertrophic growth of cells in high-glucose medium. Hypertrophy in their study was measured by an increased protein–DNA ratio. Inhibition of PI3K, either by the drug LY294002 or by transfection of a dominant-negative plasmid that inhibits the regulatory subunit (p85) of PI3K, lessened both hypertrophy and the level of p21. The increase in p21 protein was probably because of transcriptional upregulation, since the measured half-life of both p21 protein and mRNA actually decreased in cells grown in high-glucose medium, while the activity of the p21 promoter increased severalfold in high-glucose medium. Whether the hypertrophic growth was because of the effect of PI3K on p21 levels or was independent of p21 was not investigated.

It is possible to speculate, because of Chuang and colleagues' finding of transcriptional regulation of the p21 promoter,[13] that PI3K either directly or indirectly activated p21 promoter-interacting transcription factors, which were responsible for the increased level of p21 protein, resulting in hypertrophy. PI3K and Akt were previously shown to activate several transcription factors,[16] but whether these or other undetermined PI3K-dependent proteins can modulate p21 promoter activity remains to be determined. On the other hand, Akt was shown in other studies to phosphorylate p21 at a region that dissociated it from its normally inhibitory binding of PCNA, a DNA polymerase subunit necessary for DNA synthesis, while at the same time promoting p21 translocation from the nucleus into the cytoplasm.[17] These results demonstrate that the p21 and PI3K pathways intersect in at least two ways: first, by PI3K-dependent induction of p21 transcription, and second, by direct interaction between Akt and p21. Although these intersections may be sufficient to

explain the dependence of hypertrophy on PI3K, p21 has roles beyond that of cell-cycle inhibition depending on the cellular stress and environment. One of the original descriptions of p21 was as an inducer of cellular senescence,[18] and p21 deletion was shown to prolong the lifespan of telomerase-deficient cells and mice,[19] a condition associated with shortened lifespan in humans and in mice. Coincidently, p21 but not p16 (another cyclin-dependent inhibitor) was found to be involved in telomere shortening-induced senescence of human cells,[20] and transduction of p21 but not of p16 caused hypertrophy in renal proximal tubule cells.[11] Perhaps the consequences of renal hypertrophy and the fibrotic changes associated with aging are both controlled by the same PI3K/Akt/p21 pathways. These possibilities should be fruitful areas for future investigations.

In summary (Figure 1), both p21 and the PI3K/Akt pathways have been shown to be associated with cellular and organ hypertrophy. The work of Chuang *et al.*[13] reiterates this concept and at the same time shows that these pathways may be mutually interdependent. More work is needed to support these interactions and to elucidate fully the pathway of hypertrophy. At the same time, however, this work has pointed out possible intervention strategies, by PI3K/Akt inhibition, to ameliorate hypertrophy.

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Membranous nephropathy: When and how to treat

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The treatment of idiopathic membranous nephropathy is heavily debated because of wide variation in outcome. A rational treatment strategy is needed to appropriately administer conservative treatment to the low-risk group but immunosuppressive therapy to those with medium or high risk of renal deterioration. Currently, combinations of steroids with alkylating agents are best studied. Newer forms of immunosuppressive treatment are currently under study.

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Idiopathic membranous nephropathy (IMN) is a common cause of the nephrotic syndrome in adult patients. The treatment of patients with IMN has been a regular theme for debate. Today, once the diagnosis is made, symptomatic management for proteinuria and hypertension is mandated in almost all patients. The impact of these treatments alone on the natural history is expected to be positive but is difficult to delineate distinctly. This wide variation in outcome is one of the factors that have led metaanalysis and systematic reviews of this disease to reach varying conclusions about the impact of immunosuppressive treatment on patient and renal survival and on remission rate of proteinuria.

Reports of the natural history of IMN are divergent and thus have set the stage for heavy disputes on the use of immunosuppressive therapy. The spectrum varies from a relatively benign course of 65% spontaneous remission of proteinuria and

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